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Subacute Intubation Study on Rats with the Compound 2,3,7,8-Tetrachloridioxin

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Chlorine organic compounds.

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SUBACUTE INTUBATION STUDY ON RATS WITH THE COMPOUND 2,3,7,8-TETRACHLORODIOXIN

Contract No. 68-01-2224

IITRI Report L6073-12
(Final Report)

November 20, 1973 to November 19, 1974

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FOREWORD

This final report, "Subacute Intubation Study on Rats with the Compound 2,3,7,8-Tetrachlorodioxin" describes studies conducted by IIT Research Institute for the Environmental Protection Agency during the period 11-20-73 to 11-19-74. The Contract Number is 68-01-2224; the IITRI Project Number is L6073. William L. Burnam monitored the program for the EPA.

Maurice E. King, as Principal Investigator, directed the program. Administrative supervision was provided by Alan M. Shefner. Veterinary pathology services were performed by A. Ross Roesler. Dr. King was assisted in the laboratory by D. Davis, M. Dickens, A. Garner, W. Hilger and D. Tvarkunas.

Data are recorded in Logbooks C21561, C21798 and D1856.

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ABSTRACT

This report describes a 12-month study to determine the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin, (TCDD), an extremely toxic contaminant of the herbicide 2,4,5-T. Male and female rats were intubated twice weekly to provide 0, 0.1 or 1.0 μg/kg/wk of TCDD dissolved in acetone diluted with corn oil. The animals were treated for a total of 28 weeks and held for an additional 12-week recovery period. Body weight observations were made weekly throughout the entire test period. Groups of rats were killed at the end of 2,4,8,16 and 28 weeks of treatment and after 4 and 12 weeks recovery. Organ and body weights were measured and complete histopathologic examinations were made on all sacrificed rats. With this protocol TCDD was observed to be toxic but not lethal to rats. The extent of the toxic effects, which were manifested by centrilobular fatty change and increased hepatocellular turnover, were confined to the liver and were more severe in male than in female rats.

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I. INTRODUCTION

Technical grade chlorophenols and products derived from them (e.g., herbicides such as 2,4,5-T and other pesticides) have been found to contain chlorinated dibenzo-p-dioxins as contaminants. The herbicide 2,4,5-T has been widely used for some time to control brush and weeds on rangelands, pastures, and rights-of-way. In addition, its use as a defoliating agent during the Vietnam conflict has contributed to the overall quantity in the environment. The contaminants, which may be formed as byproducts in the manufacture of chlorinated phenols and carried over to their products, are among the most toxic substances known. One of these compounds, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), has caused chloracne, a severe skin disease, among workers engaged in the manufacture of chlorophenols, it is teratogenic and/or embryotoxic to all species tested to date. Although the herbicide is slowly degraded in the soil, the impurity TCDD, is quite stable and its concentration can increase over a period of time. Because of the persistance of TCDD and the possibility of its entering the food chain, preliminary studies were initiated to determine the effects of subacute oral administration of TCDD to rats. Male and female young adult rats were intubated with TCDD at two dosage levels for 28 weeks then allowed to recover for 12 weeks. Animals were killed at intervals during the test and recovery periods and their organs examined histopathologically. Growth rate studies were made. Because of the highly toxic nature of TCDD, all procedures were carried out in specialized laboratories and hoods and the animals were housed in individually ventilated and filtered cages.

II. MATERIALS AND METHODS

The TCDD used in this study was from a quantity synthesized by the IITRI Chemistry Division under National Cancer Institute Contract No. NO1-CP-12338. The use of the material was approved by Dr. H. F. Kraybill, Scientific Coordinator for Environmental Carcinogenesis, NCI. A 500 µg/ml stock solution was prepared by dissolving 50 mg TCDD in 100 ml acetone. Successive 10-fold dilutions were then made with acetone and corn oil to provide solutions containing 0.5 and 0.05 µg/ml.

Each treatment group consisted of 35 male and 35 female Sprague-Dawley weanling rats that weighed between 100 and 150 grams at the beginning of the study. The TCDD was administered by oral intubation in a 9:1 mixture of corn oil and acetone; the control rats received only the corn-oil/acetone. Each animal was weighed weekly and the volume of TCDD solution administered was adjusted for the proper dosage level. For example, when 0.2 ml of each solution was given to 200-g rats twice weekly, doses of 1 and 0.1 µg/kg/wk, which are roughly equivalent to dietary levels of 3 and 0.3 parts per billion, were obtained.

The rats were treated Tuesday and Thursday of each week for 28 weeks then held for an additional 12-week recovery period during which no treatment was given. Three animals of each sex from each dosage groups were killed at the end of 2,4,8 and 16 weeks of treatment and 10 from each group at the end of the 28 week treatment period. Three rats from each treatment group were killed at 4 and at 12 weeks following cessation of treatment. At necropsy, the rats were examined for gross abnormalities and the weights of the liver, spleen, gonads, kidneys, heart, brain, and lungs were determined. Portions of specific organs were preserved in formalin and processed for histopathologic examination. The remaining portions were frozen and submitted to the Pesticide Monitoring Laboratory, Bay St. Louis Mississippi, for TCDD

analysis. Because the animals killed at 2 and 4 weeks were too small to provide adequate samples for tissue analysis, additional rats were placed on test, killed on this schedule and the corresponding tissues from each group pooled for TCDD analysis. Live rats and blood samples from the 28-week treatment groups and the 12-week recovery groups were sent to the Primate and Pesticide Effects Laboratory, Research Triangle Park, North Carolina, for blood chemistry and enzyme studies.

Animals were anesthetized with sodium pentobarbitol and killed by intracardial exsanguination. Food, but not water, had been withheld for the previous 16 hr. After gross examination and weighing, the tissues from each animal were fixed for 48 hr in buffered neutral 10% formalin, dehydrated, embedded in Paraplast, sectioned at 4 to 6 μm and stained with hematoxylin and eosin (H & E). After examination of the H & E sections, special stains including McManus Periodic Acid Schiff (PAS), Gomori's method for iron, Oil Red O method for lipofuscin, and the AFIP method for lipofuscin and ceroid were applied to selected sections. Standard formalin fixed liver of two rats from the 28-week sacrifice (a male control and a high-dose male in which extensive hepatocellular vacuolation had been observed) were prepared for electron microscopy. The liver was diced in 1-2 mm cubes, washed for 2 hr in phosphate buffer, fixed in phosphate-buffered 1% OsO_4 for 1 hr, dehydrated and embedded in Spurr's media. Sections were cut at 1 μm on a Sorvall ultramicrotome and, after examination by light microscopy, selected areas were cut at 40-50 nm, placed on uncoated copper grids, and examined in an RCA EMU-4B at 75 kv.

The tissues sectioned for H & E staining were:

- Esophagus
- Stomach (glandular and aglandular)
- Small intestine
- Cecum
- Colon
- Liver (usually 2 sections)
- Kidneys (½ of each)
- Spleen
- Salivary gland
- Heart
- Aorta
- Lymph node
- Skeletal muscle
- Trachea
- Lung (5 lobes)
- Thymus
- Pancreas
- Urinary bladder
- Pituitary gland
- Adrenal glands (2)
- Ovary/testis (1)
- Seminal vesicle
- Prostate/uterus
- Bone marrow (Sternum)
- Thyroids (2)
- Parathyroids (2)
- Brain (2 sections)
- Spinal cord
- Peripheral nerve (sciatic)
- Optic nerve (2)
- Harderian glands (2)
- Eyes (2)

III. RESULTS AND DISCUSSION

A. Body and Organ Weights

Changes in body weight with time for TCDD-treated and control groups are shown in Figure 1. The growth rates of male and female groups at both levels of TCDD were less than those of the control groups that received only the corn-oil acetone mixture. Statistical analyses of the data are summarized in Appendix A. Tables A1 and A3 summarize the results of an analysis of variance of male and female growth rates during the treatment and recovery periods. In Table A1, the differences in growth rates for the three male groups are shown to be more significant during the treatment period than during the recovery period. A similar situation is seen in Table A3 for the female growth rates but the significance for both periods is less than the analogous results for males.

Tables A2 and A4 list the mean growth rates with their confidence limits and the results from application of Duncan's multiple range test (MRT) to the data. The test is based on the Student-t statistic and was used to determine if the values are significantly different at the 95% level. The letters a, b, and c indicate if there is a significant difference: a is significantly different from b and c; b is significantly different from a and c; c is significantly different from a and b; ab indicates that the value does not differ significantly from either a or b.

> For example: In Table A12, page A8, the values for female liver weights are compared. At the 8-week interval, the liver weights for the three doses are significantly different from each other. Thus, MRT notations a, b, and c are listed for the control, 0.1 µg and 1.0 µg groups. However, at the 16-week interval, the control value is significantly different from that of the 1.0 µg group but the 0.1 µg value is not significantly different from the control or the 1.0 µg group. Therefore, the MRT notations are a, ab, and b for control, 0.1 and 1.0 µg groups.

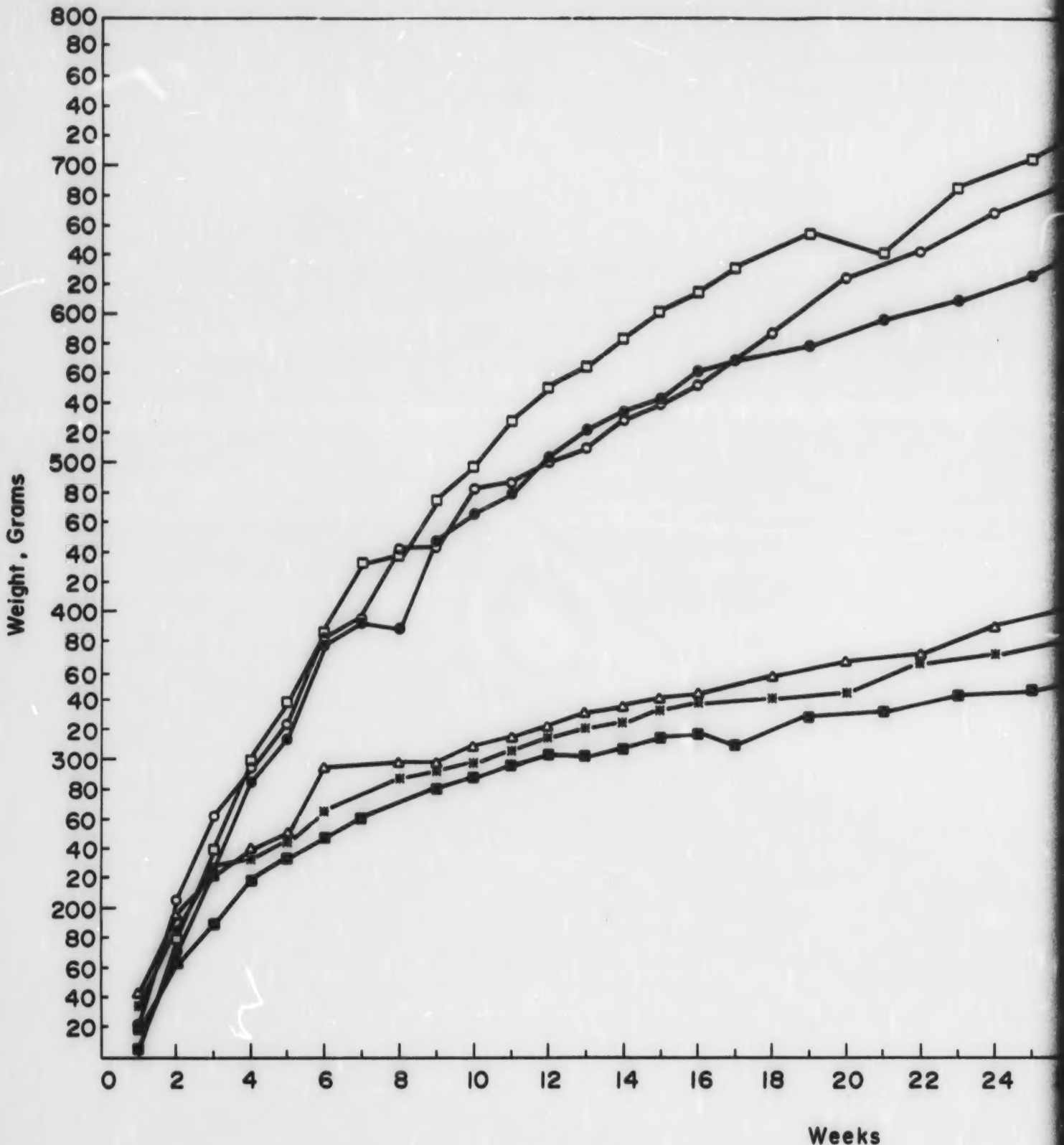
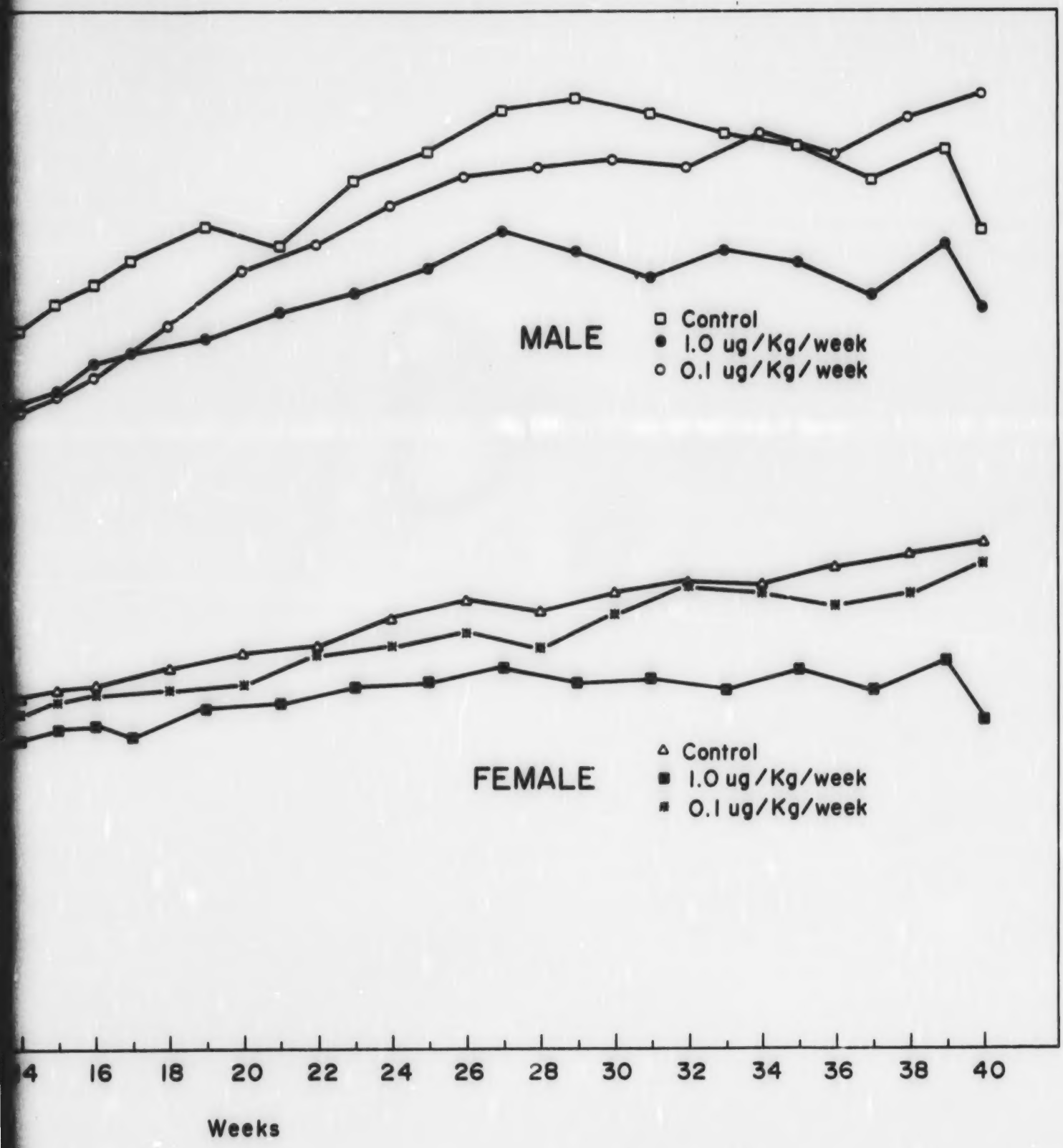


FIGURE 1 AVERAGE WEIGHT OF TREATED MA



E WEIGHT OF TREATED MALE AND FEMALE RATS

The results in Table A2 show that there is a significant difference in the growth rate between male controls and the two treated groups during both test periods whereas Table A4 shows a significant difference between female controls and both treated groups during the treatment period but only for the 1.0 μg/wk group during the recovery period.

Table 1 lists the average body weights and the organ-to-body weight ratios for the animals killed at the various sacrifice intervals. Generally, the organ weight as percent of body weight for both control and treated rats decreased as the animals became older. Although variations in these values are seen for all organs, the differences between controls and treated rats appear to be most consistent for the liver. The sacrifice body weights and the organ weight percentages were statistically analyzed and summary tables for the analysis of variance and the multiple range test for the mean values at each sacrifice interval are given in Appendix A. The analyses compared the results for the different treatment groups as well as the sacrifice intervals and the interaction between the treatments and the sacrifice intervals. Tables A5 and A7 show for males and females that, in addition to a significant difference in sacrifice body weight of the treatment groups for the complete test period, values for the weights at the sacrifice intervals and also the interaction between sacrifice intervals and group treatments are significantly different. Tables A6 and A8, however, show that a consistent significant difference in the mean body weights does not occur until the 28-week sacrifice for both sexes although the 1.0 μg female mean value was significantly lower at the 8-week sacrifice.

TABLE 1

EFFECT OF TWICE WEEKLY INTUBATION OF TCDD ON RATS

Treatment Group	Week of Test	Body Weight (g)	Percent of Body Weight						
			Liver	Spleen	Gonads	Kidneys	Heart	Brain	Lungs
Male	2	226	5.47	0.49	1.25	1.17	0.48	0.71	
Control	4	333	4.09	0.23	1.03	0.86	0.37	0.67	
	8	430	4.34	0.34	0.93	0.79	0.34	0.52	0.77
	16	642	3.72	0.15	0.55	0.68	0.26	0.34	0.48
	28*	726	2.70	0.12	0.46	0.57	0.26	0.31	0.47
	4**	750	2.87	0.14	0.35	0.59	0.27	0.30	0.45
	12	606	2.48	0.13	0.56	0.59	0.28	0.36	0.47
Males	2	231	5.80	0.38	1.09	1.18	0.44	0.84	
@ 0.1 µg/kg	4	326	5.38	0.29	0.99	0.95	0.41	0.67	
	8	441	4.53	0.20	0.71	0.84	0.31	0.48	0.72
	16	583	4.09	0.14	0.57	0.65	0.25	0.28	0.59
	28*	685	3.66	0.13	0.53	0.61	0.25	0.32	0.56
	4**	649	2.72	0.14	0.42	0.60	0.28	0.35	0.50
	12	760	3.71	0.14	0.46	0.63	0.24	0.29	0.52
Males	2	213	6.77	0.29	1.12	1.10	0.48	0.91	
@ 1.0 µg/kg	44	334	5.20	0.34	0.90	0.87	0.36	0.65	
	8	368	4.92	0.20	0.64	0.82	0.31	0.63	0.84
	16	589	4.17	0.15	0.50	0.67	0.36	0.36	0.67
	28*	676	3.25	0.15	0.56	0.61	0.28	0.35	0.52
	4**	618	3.72	0.14	0.59	0.62	0.27	0.36	0.47
	12	566	2.66	0.16	0.55	0.54	0.27	0.31	0.45
Female	2	199	5.08	0.28	0.07	0.95	0.42	0.97	
Control	4	231	4.26	0.25	0.14	0.87	0.39	1.01	
	8	293	3.76	0.20	0.06	0.78	0.36	0.58	0.87
	16	338	3.47	0.18	0.04	0.73	0.29	0.60	0.86
	28*	408	3.36	0.16	0.05	0.62	0.29	0.49	0.58
	4**	424	3.09	0.14	0.03	0.66	0.27	0.44	0.50
	12	468	3.50	0.13	0.03	0.57	0.25	0.44	0.47
Females	2	182	5.15	0.32	0.12	1.03	0.44	1.01	
@ 0.1 µg/kg	4	238	4.54	0.24	0.08	0.74	0.32	0.8	
	8	289	4.62	0.22	0.04	0.76	0.32	0.65	0.84
	16	325	3.60	0.17	0.05	0.68	0.32	0.60	0.71
	28*	356	3.82	0.18	0.06	0.73	0.34	0.56	0.78
	4**	442	3.19	0.14	0.03	0.64	0.28	0.51	0.59
	12	440	3.86	0.14	0.03	0.67	0.31	0.44	0.64
Females	2	158	5.09	0.33	0.11	1.02	0.46	1.08	
@ 1.0 µg/kg	4	219	5.22	0.33	0.11	0.75	0.39	0.92	
	88	209	5.23	0.30	0.07	1.04	0.38	1.21	1.23
	16	324	4.05	0.18	0.05	0.73	0.30	0.65	0.77
	28*	347	3.61	0.18	0.08	0.72	0.36	0.57	0.71
	4**	369	3.87	0.16	0.04	0.73	0.31	0.53	0.68
	12+	327	4.27	0.29	0.05	0.82	0.38	0.64	0.65

* 28-Week Data on 10 rats, other date on 3 rats.

** 4 and 12 weeks after cessation of treatment.

\+ group contained 4 rats, 1 with apparent mammary tumor.

The liver data were analyzed for the complete 40-week test period and also separately for the 28-week test and 12-week recovery periods. Tables A9 and A11 indicate that the values for sacrifice intervals and treatment groups as well as the interactions are significantly different over the complete test period for both sexes. When the treatment and recovery periods are analyzed separately, however, the male liver values are no longer significantly different for the recovery period and the female values are at a lower probability level than was found for the complete test period. These results are compatible with the histopathologic observations.

As the summary tables for the other organ weight percentages (Appendix A) show, in all cases there is a significant difference in the values for the sacrifice intervals. The values for the different treatment groups and the interactions are, for the most part, nonsignificant and, in those cases in which there is significance, it is usually at a low probability level. In view of the histopathologic findings, the statistical differences observed for these organs are not likely to be biologically significant but merely reflect the normal change in organ weight ratios as the animals become older.

B. Histopathologic Observations

1. Incidental Findings

About 45% (38/84) of all male animals had a mild to marked multifocal chronic myocarditis, a common lesion associated with aging in the male rat. About 40% (32/85) of the females had mineralized deposits, probably calcium salts, in their renal tubules usually near the cortico-medullary junction. There appeared to be neither a positive nor negative correlation between these observations and TCDD administration. As the animals were maintained individually in filter top cages, the incidence of mild to moderate chronic murine and chronic interstitial pneumonias was quite low and not considered a factor affecting the results. The only other incidental findings of note are those of a nephroblastoma in a control male at 28 weeks (animal 234) and a mammary adenocarcinoma in a high-dose female (animal 214) that was killed at the end of the 40-week test period.

2. TCDD-Related Observations

Histopathologic changes that could be attributed to TCDD administration were restricted to the liver at 28 weeks of treatment and beyond; all animals killed at 2 through 16 weeks were essentially normal. As there was no sacrifice between 16 and 28 weeks and there was apparent recovery in some animals after cessation of TCDD administration at 28 weeks, we cannot definitely determine if the hepatotoxicity is progressive or accumulative. However, as a result of other TCDD animal experiments conducted in this laboratory, we are certain that had a sacrifice been conducted at 22 to 24 weeks, the results would confirm the progressive nature of TCDD-induced hepatic toxicity. Tables 2-7, abstracted from the complete list of histologic findings observed in the rat livers of the final three sacrifices (Appendix B), enable a comparison of the various dosages. Figures 2-9 are photomicrographs of livers from the various groups illustrating some of the hepatic changes noted.

Table 2 lists the number of animals that were observed to have essentially normal livers. Many animals counted as "normal" in this table had a very minor change or two, which was considered equivocal or within normal limits. It can be readily seen that the males were more adversely affected than the females and were slower to recover.

Table 3 lists and grades the extent of the most significant alteration observed in the H & E sections, hepatocellular vacuolation, which usually began in the centrilobular region (Figures 4 and 5). By examination of 1 μm and 40-50 nm sections of post-OsO_4-fixed, epoxy-embedded liver material with light and electron microscopy, these vacuoles were seen to contain lipid droplets that were often larger than the nuclei (Figures 6 and 7), constituting a diagnosis of fatty change. Even though the resolution is poor as the tissue was not originally fixed for electron microscopy, membrane whorls probably representing

Table 2

NUMBER OF ANIMALS WITH ESSENTIALLY NORMAL* LIVERS

Treatment Group	Number of animals with normal livers after 28 Weeks	4-week recovery	12-week recovery
Male control	8/10	3/3	3/3
Males @ 0.1 µg/kg	3/10	2/3	2/3
Males @ 1.0 µg/kg	1/10	0/3	2/3
Female Control	9/10	3/3	3/3
Females @ 0.1 µg/kg	7/9**	3/3	3/3
Females @ 1.0 µg/kg	2/10	1/3	1/4

* See Page 15, paragraph 2 for explanation of "normal".

**Liver of one animal lost.

Table 3

HEPATOCELLULAR FATTY CHANGE *

Treatment Group	28 Weeks					4-Weeks Recovery					12-Weeks Recovery				
	N	S	Mi	Mo	Ma	N	S	Mi	Mo	Ma	N	S	MI	Mo	Ma
Male Control	7/10	3/10	--	--	--	2/3	1/3	--	--	--	3/3	--	--	--	--
Males @ 0.1 µg/kg	2/10	1/10	1/10	5/10	1/10	2/3	1/3	--	--	--	2/3	--	--	1/3	--
Males @ 1.0 µg/kg	0/10	1/10	2/10	5/10	2/10	--	1/3	1/3	1/3	--	2/3	--	--	1/3	--
Female Control	10/10	--	--	--	--	3/3	--	--	--	--	3/3	--	--	--	--
Females @ 0.1 µg/kg	4/9	4/9	1/9	--	--	3/3	--	--	--	--	3/3	--	--	--	--
Females @ 1.0 µg/kg	1/10	4/10	4/10	1/10	--	1/3	1/3	--	1/3	--	1/4	3/4	--	--	--

*N: None
S: Slight: random hepatocyte containing a solitary, large lipid droplet-equivocal
Mi: Mild: several centrilobular hepatocytes contain lipid
Mo: Moderate: most centrilobular hepatocytes contain lipid
Ma: Marked: all centrilobular and some midzonal hepatocytes contain lipid

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an increase in endoplasmic reticulum (ER) turnover can also be seen in the lipid inclusions and within the Kupffer cell in Figure 7. Although the quality of this electron micrograph does not allow differentiation between smooth and rough ER, we feel that this observation is consistent with hepatic smooth ER proliferation reported in association with chlorinated aromatic hydrocarbon intoxication (D. H. Norback and J. R. Allen, Environ. Health Perspective, p. 137, April 1972).

In the male, but not female animals, the control hepatocytes often contained a number of small lipid vacuoles; hence this observation was considered normal for the males. Even in controls a random hepatocyte was occasionally seen to contain a solitary, large lipid vacuole. This was called "slight" in the table and is probably normal for the males, but likely threshold or borderline for the females. When several centrilobular hepatocytes were seen to contain one to several large lipid droplets, the lesion was designated "mild". Fatty change involving most of the centrilobular hepatocytes was considered "moderate"; if all centrilobular and many midzonal hepatocytes were affected, the lesion was graded "marked". No animal in this experiment was graded "severe", which would indicate involvement of over half of the liver cells. In general, there was little or no inflamatory response even in the livers with marked fatty change. From the table we see that this lesion was more pronounced in the males than the females. Considering the number of affected animals at each dose level there appears to be a slight dose response in the males. The distribution of the severity of the lesion however, is such that, on an individual animal basis, it is not possible to discern histologically whether a given individual was from the high or low dose group.

Table 4 tabulates the incidence of hepatocellular loss and necrosis. In most cases this was a subtle change; there was an occasional centrilobular cell undergoing karyorrhexis, or the immediate area around the central vein was devoid of hepatocytes, leaving a dilated, empty space, or a space containing a few red blood cells.

Tables 5 and 6 cite the occurrence of various parameters that are usually associated with cell death or repair. Double nucleated hepatocytes are common in most normal rodents and were seen in all the livers, control and treated, in this experiment (Figure 3). On rare occasion, the number of double nucleates may have been slightly increased in some of the test animals, an indication of repair, but for the most part this observation was considered an insignificant parameter. There were, however, a few treated animals in which an occasional hepatocyte with four or more nuclei was seen, and this was considered significant (Figure 9). In the normal rat liver the cell and nuclear size tends to be almost uniform throughout the lobule (Figures 2 and 3) although there often is a variation in staining that is due to such factors as interval between last meal and death, and interval between death and fixation. In treated animals, especially the females, the cell and nuclear size was often observed to be larger by a factor of as much as two toward the peripheral and midzonal regions of the lobule relative to those cells in the region of the central vein. These enlarged cells often compressed the hepatic cords and sinusoids, leading to a subtle alteration in the normal architecture. The lobule appeared to be undergoing repair from the peripheral and midzonal regions toward the center. Interestingly, no mitotic activity was noted in any of the livers as opposed to what is seen in the rat liver following acute CCl_4 intoxication.

Table 4

HEPATOCELLULAR NECROSIS/CELL LOSS

Treatment Group	Severity of Cell Loss* after 28 Weeks			4-Weeks Recovery			12-Weeks Recovery		
	N	S	M	N	S	M	N	S	M
Male Control	10/10	4/10	1/10	--	--	--	--	--	--
Males @ 0.1 µg/kg	5/10	1/10	--	3/3	--	--	3/3	--	--
Males @ 1.0 µg/kg	9/10	1/10	--	3/3	--	--	3/3	--	--
Female Control	10/10	--	--	3/3	--	--	3/3	--	--
Females @ 0.1 µg/kg	8/9	1/9	--	3/3	--	--	3/3		
Females @ 1.0 µg/kg	7/10	1/10	2/10	2/3	--	1/3	4/4	--	--

*N: None; S: Slight; M: Mild

This observation mainly pertains to centrilobular hepatocytes, and is a difficult observation to decide upon in the case of those males with extensive centrilobular fatty change.

Table 5

HEPATOCELLULAR MULTINUCLEATION, VARIATION IN CELL/NUCLEAR SIZE AND/OR DISTORTED ARCHITECTURE

Treatment Group	Severity* of Multinucleation after 28 Weeks		4-Weeks Recovery		12-Weeks Recovery		
	N	S	N	S	N	S	M
Male Control	10/10	--	3/3	--	3/3	--	--
Males @ 0.1 µg/kg	10/10	--	3/3	--	3/3	--	--
Males @ 1.0 µg/kg	10/10	--	3/3	--	2/3	--	1/3
Female Control	10/10	--	3/3	--	3/3	--	--
Females @ 0.1 µg/kg	7/9	2/9	3/3	--	3/3	--	--
Females @ 1.0 µg/kg	9/10	1/10	2/3	1/3	--	3/4	1/4

*N: None
S: Slight
M: Mild

Table 6

EXCESSIVE RANDOM HEPATOCYTES WITH
HYPERCHROMATIC NUCLEI AND CYTOPLASM

Treatment Group	28 Weeks	4-Weeks Recovery	12-Weeks Recovery
Male Controls	2/10	0/3	3/3
Males @ 0.1 µg/kg	0/10	1/3	0/3
Males @ 1.0 µg/kg	1/10	0/3	1/3
Female Controls	1/10	0/3	0/3
Females @ 0.1 µg/kg	3/9	2/3	0/4
Females @ 1.0 µg/kg	3/10	0/3	0/4

This, coupled with the observation of some abnormal livers even after 12 weeks withdrawal from treatment, indicates that recovery from TCDD insult is a slow process.

Occasional, randomly located individual cells or pairs of adjacent cells were seen in most test and control animals (Figure 3) with cytoplasm and nuclei which appeared hyperchromatic with H & E staining. For the most part, this was interpreted as one of the normal growth/repair functions of the liver. In a few animals, as indicated in Table 6, the number of these cells was deemed excessive and tabulated accordingly. It does not appear that this observation is significant for the males but it apparently is indicative of active repair in the females.

Occasional macrophages containing a brown pigment in H & E section were seen in many animals, including controls. There was, however, often an increase in the number of these pigment-containing macrophages (Figure 8), usually in the centrilobular region, in the females (Table 7). Special stains, including the Oil Red O and AFIP Method for lipofuscin and Giomori's Method for iron, were applied to selected female livers to characterize this pigment. The first two stains were negative for lipofuscin (ceroid) whereas the last was positive for iron: this indicates that the pigment is predominantly hemosiderin-related and agrees with a recent German study (personal communication, Dr. H. L. Richardson) which concludes that pigment found in the livers of humans exposed to TCDD is a "pre-hemosiderin". There were no histologic observations that suggest a reason for the apparent increase in red blood cell breakdown in the females.

Table 7

INCIDENCE OF PIGMENTED MACROPHAGES IN FEMALES

Treatment Group	28-Weeks			Severity* after 4-Weeks Recovery				12-Weeks Recovery		
	N	S	Mo	N	S	Mo	Ma	N	S	M
Control	10/10	--	--	3/3	--	--	--	2/3	1/3	--
0.1 g/kg	8/9	1/9	--	3/3	--	--	--	1/3	2/3	--
1.0 g/kg	5/10	4/10	1/10	1/3	--	1/3	1/3	--	3/4	1/4

*N: Normal
S: Slight
Mo: Moderate
Ma: Marked

IV. CONCLUSIONS

1. Intragastric administration of TCDD to male and female Sprague-Dawley rats at the rate of 0.1 or 1.0 μg/kg/week for 28 weeks is toxic but not lethal.

2. The toxic effects of TCDD are restricted to the liver but there is significant individual variation in severity.

3. The most extreme change observed as a result of TCDD administration was centrilobular fatty change and in no case was this severe. Other toxic manifestations noted, while real, usually affected only a few cells and, considering the total mass of the liver, were subtle or not significant.

4. The toxicity is delayed as only equivocal hepatic histologic changes were observed after 16 weeks of treatment but marked alterations were present after 28 weeks of treatment.

5. The lesion resulting from this 28-week relatively low-level exposure to TCDD is reversible with time, but even 12 weeks was not a sufficient recovery period for some animals.

6. The females, but not the males, were dose-responsive based on histologic observations during both treatment and recovery.

7. There was a definite sex-related response, with the males developing a higher incidence of more extreme histologic response. The females tended to respond in a more subtle manner, such as altered architecture, variation in cell and nuclear size and multinucleation.

8. Fatty change, the most significant lesion, suggests that impaired hepatic lipid transport and/or metabolism is a significant mechanism in the TCDD intoxication observed with this protocol.

DESCRIPTION OF FIGURES

Figure 2: Normal Liver, Male Rat 40x.

Figure 3: Normal Liver, Female Rat. Two of the several binucleated hepatocytes present are indicated by the arrows. Two pairs of cells with hyperchromatic nuclei and cytoplasm are indicated by (►); central vein (*). 170x.

Figure 4: Liver, Male given 1.0 g TCDD/kg/wk for 28 weeks. Some of the numerous areas of moderate centrilobular fatty change (*); portal triad (→). 40x.

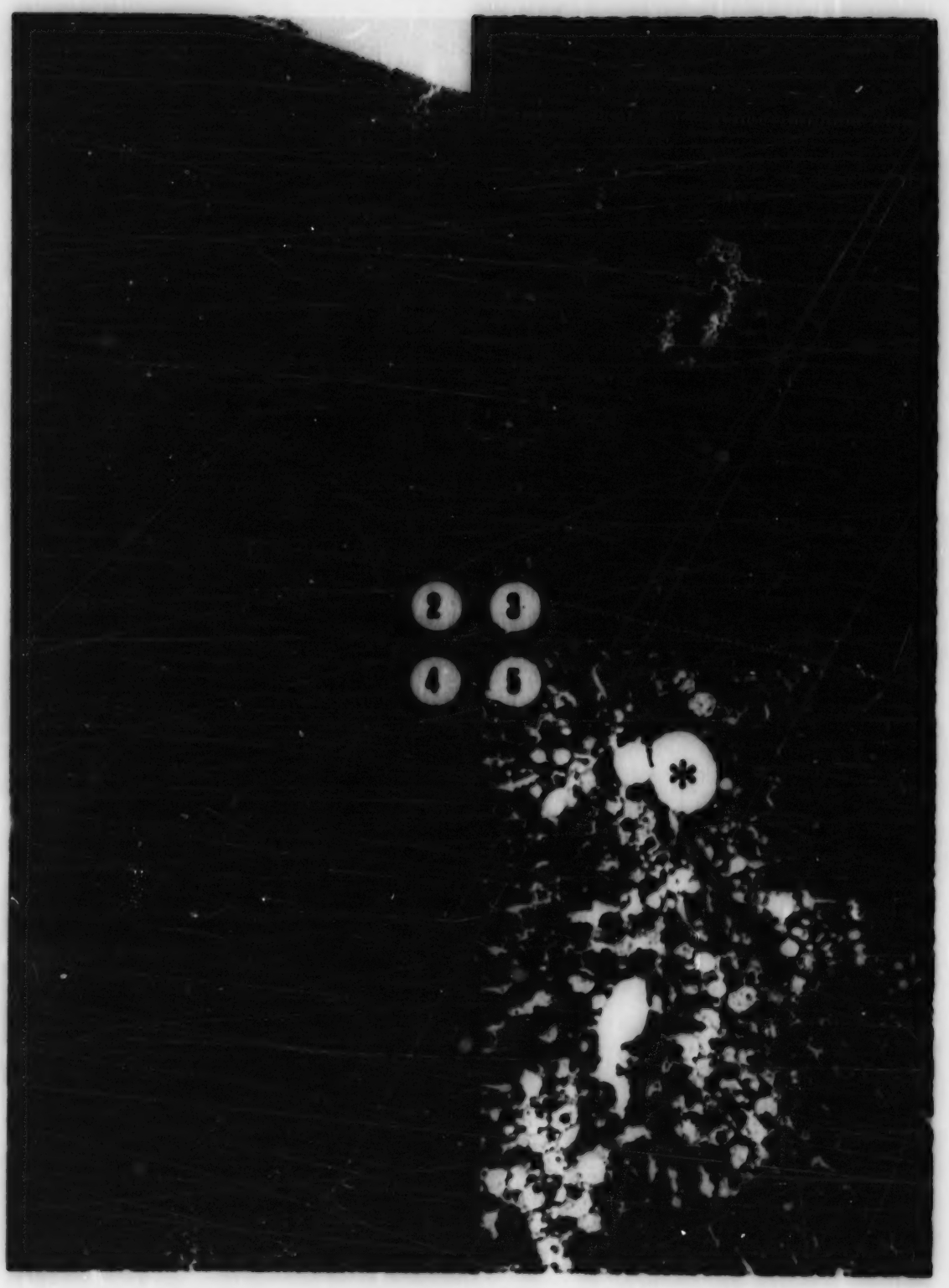
Figure 5: Higher power of Figure 4, centrilobular fatty change; central vein (*). 170x.

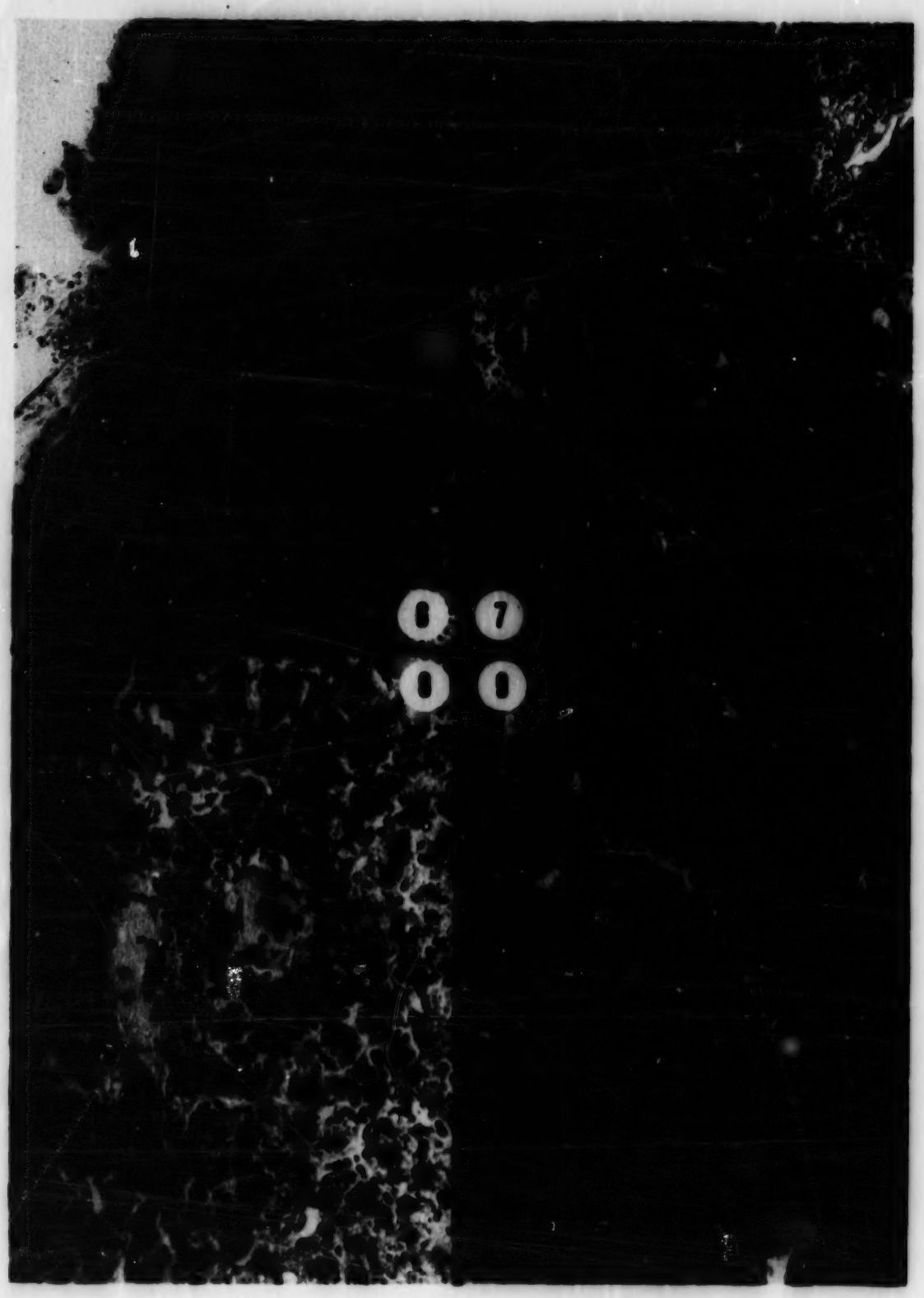
Figure 6: Liver, Male given 1.0 µg TCDD/kg/wk for 28 weeks. Toluidine Blue stained, 1 µm epoxy embedded section. The large black cytoplasmic bodies are abnormal hepatocellular lipid accumulations stained with OsO_4. 170x.

Figure 7: Electron micrograph taken from lower left portion of Figure 6. Compare size of cytoplasmic lipid droplets (L) with size of hepatocyte nucleus (N), red cell (R) in sinusoid, and a Kupffer cell (K). The lipid inclusions at the lower left and center also contain membrane whorls which are remnants of endoplasmic reticulum. 3400x.

Figure 8: Liver, Female given 1.0 µg TCDD/kg/wk for 28 weeks then allowed to recover for 4 weeks withdrawal. (*) indicates central vein, arrows point to pigment filled macrophages. 275x.

Figure 9: Liver, Female given 1.0µ TCDD/kg/wk for 28 weeks plus 4 weeks withdrawal with a multinucleated hepatocyte (→) close to the central vein (*).

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APPENDIX A

STATISTICAL ANALYSIS OF GROWTH AND ORGAN WEIGHT DATA

Table A1

ANALYSIS OF VARIANCE, MALE GROWTH RATE

(a) Treatment Period 2 → 28 Weeks

Source	Degrees of Freedom DF	Sum of Squares SS	Mean Squares MS	Ratio F	Probability P
Treatment	2	133.02	66.509	11.52	<0.0005
Residual Error	63	363.80	5.775		
Total	65	496.82			

(b) Recovery Period 28 → 40 Weeks

Source	DF	SS	MS	F	P
Treatment	2	96.23	48.115	4.46	<0.05
Residual Error	12	129.58	10.798		
Total	14	225.81			

Table A2

SUMMARY OF MEAN MALE GROWTH RATE AND MULTIPLE RANGE TEST*

(a) Treatment Period

Dose	Number of Observations	Mean g/Wk	Multiple Range Test* MRT	Confidence Limits 95%	Confidence Limits 99%
0.0	23	19.614	a	±1.002	±1.332
0.1	20	16.824	b	±1.074	±1.429
1.0	23	16.494	b	±1.002	±1.332

(b) Recovery Period

0.0	5	-4.925	a	±3.274	±4.657
0.1	5	0.252	b	±3.274	±4.657
1.0	5	0.625	b	±3.274	±4.657

* Multiple Range Test: a is significantly different from b and c
b is significantly different from a and c
c is significantly different from a and b
ab indicates there is no significant difference between the mean of a and the mean of b.
(In all cases, "Significantly" means significant at the 95% or greater level).

Table A3

ANALYSIS OF VARIANCE, FEMALE GROWTH RATE

(a) Treatment Period 2 → 28 Weeks

Source	Degrees of Freedom DF	Sum of Squares SS	Mean Squares SS	Ratio F	Probability P
Treatment	2	9.808	4.9040	3.219	<0.05
Residual Error	65	99.024	1.5234		
Total	67	108.832			

(b) Recovery Period 28 → 40 Weeks

Source	DF	SS	SS	F	P
Treatment	2	30.089	15.044	3.82	<0.10
Residual Error	12	47.202	3.933		
Total	14	77.291			

Table A4

SUMMARY OF MEAN FEMALE GROWTH RATE AND MULTIPLE RANGE TEST

(a) Treatment Period

Dose	Number of Observations	Mean g/Wk	MRT	Confidence Limits 95%	Confidence Limits 99%
0.0	23	7.516	a	+0.514	+0.684
0.1	23	6.828	b	∓0.514	∓0.684
1.0	22	6.630	b	∓0.526	∓0.699

(b) Recovery Period

0.0	5	3.438	a	+1.976	+2.811
0.1	5	3.116	a	∓1.976	∓2.811
1.0	5	0.286	b	∓1.976	∓2.811

Table A5

TWO-WAY ANALYSIS OF VARIANCE, BODY WEIGHTS OF MALES AT SACRIFICE

Source	DF	SS x 10^{-3}	MS x 10^{-3}	F	P
Week	6	2434.20	405.69	154.60	<0.0005
Treatment	2	37.20	18.60	7.09	<0.005
W*T	12	85.31	7.11	2.71	<0.005
Error	63	165.32	2.62		
Total	83	2722.00			

Table A6

SUMMARY, WEIGHTS OF MALES AT SACRIFICE AND MULTIPLE RANGE TEST

Week	Number of Observations	Body Weight (g) for Dose			MRT for Dose:			Confidence Limits	
		0.0	0.1	1.0	0	0.1	1.0	95%	99%
2	3	206.0	231.0	213.0	a	a	a	±59.1	±78.0
4	3	333.0	325.7	334.3	a	a	a	±59.1	±78.0
8	3	430.3	441.3	367.7	a	a	a	±59.1	±78.0
16	3	642.3	583.0	589.0	a	a	a	±59.1	±78.0
28	10	726.4	685.0	676.0	a	ab	b	±32.4	±42.7
32	3	750.3	649.0	618.0	a	b	b	±59.1	±78.0
40	3	605.7	759.7	566.0	b	a	b	±59.1	±78.0
Mean	28	547.4	565.0	529.4	ab	a	b	±19.3	±25.5

Table A7

TWO-WAY ANALYSIS OF VARIANCE, BODY WEIGHTS OF FEMALES AT SACRIFICE

Source	DF	SS x 10^{-3}	MS x 10^{-3}	F	P
Week	6	505.64	842.74	105.38	<0.0005
Treatment	2	48.90	244.50	30.57	<0.0005
W*T	12	29.31	24.42	3.05	<0.005
Error	63	50.38	799.68		
Total	83	634.23			

Table A8

SUMMARY, WEIGHTS OF FEMALES AT SACRIFICE AND MULTIPLE RANGE TEST

Week	Number of Observations	Body Weight (g) for Dose 0.0	0.1	1.0	MRT for Dose: 0	0.1	1.0	Confidence Limits 95%	99%
2	3	199.3	182.3	158.3	a	a	a	±32.6	±43.1
4	3	231.3	237.7	219.0	a	a	a	±32.6	±43.1
8	3	293.3	289.3	208.7	a	a	b	±32.6	±43.1
16	3	338.7	325.3	324.3	a	a	a	±32.6	±43.1
28	10	408.3	356.0	346.8	a	b	b	±17.9	±23.6
32	3	424.0	442.3	369.0	a	a	b	±32.6	±43.1
40	3	468.3	440.3	333.7	a	a	b	±32.6	±43.1
Mean	28	355.3	332.6	296.7	a	b	c	±10.7	±14.1

Table A9

TWO-WAY ANALYSIS OF VARIANCE, MALE LIVER AS PERCENT OF BODY WEIGHT

(a) Total Experimental Period

Source	DF	SS	MS	F	P
Week	6	77.507	12.9180	66.13	<0.0005
Treatment	2	9.771	4.8855	25.01	<0.0005
W*T	12	7.273	0.6061	3.10	<0.005
Error	63	12.307	0.1953		
Total	83	106.860			

(b) Treatment Period

Source	DF	SS	MS	F	P
Week	4	61.168	15.2920	68.53	<0.0005
Treatment	2	8.848	4.4240	19.83	<0.0005
W*T	8	3.779	0.4724	2.17	<0.10
Error	51	11.381	0.2231		
	65	85.177			

(c) Recovery Period

Source	DF	SS	MS	F	P
Week	2	0.45795	0.22897	1.56	n.s.
Treatment	2	5.43035	2.71518	18.55	<0.0005
W*T	4	3.73600	0.93400	6.38	<0.0005
Error	39	5.70780	0.14635		
Total	47	15.33200			

Table A10

SUMMARY, MALE LIVER WEIGHTS AND MULTIPLE RANGE TEST

Week	Number of Observations	Body Weight (%) for Dose 0.0	0.1	1.0	MRT for Dose: 0	0.1	1.0	Confidence Limits 95%	99%
2	3	5.567	5.810	6.767	a	a	b	±0.510	±0.678
4	3	4.094	5.377	5.202	a	b	b	∓0.510	∓0.678
8	3	4.338	4.528	4.921	a	a	b	∓0.510	∓0.678
16	3	3.730	4.086	4.177	a	a	a	∓0.510	∓0.678
28	10	2.695	3.666	3.249	a	c	b	∓0.279	∓0.372
32	3	2.875	2.718	3.728	a	a	b	∓0.510	∓0.678
40	3	2.477	3.709	2.662	a	b	a	±0.510	±0.678
Mean	28	3.388	4.119	3.532	a	b	a	±0.167	±0.221

Table A11

TWO-WAY ANALYSIS OF VARIANCE, FEMALE LIVER AS PERCENT OF BODY WEIGHT

(a) Total Experimental Period

Source	DF	SS	MS	F	P
Week	6	27.3120	4.5520	38.26	<0.0005
Treatment	2	4.3150	2.1575	18.13	<0.0005
W*T	12	3.5225	0.2935	2.47	<0.025
Error	63	7.4955	0.1190		
Total	83	42.6450			

(b) Treatment Period

Source	DF	SS	MS	F	P
Week	4	22.7540	5.6884	46.19	<0.0005
Treatment	2	3.2795	1.6398	13.32	<0.0005
W*T	8	3.1131	0.3891	3.16	<0.01
Error	51	6.2804	0.1231		
Total	65	35.4270			

(c) Recovery Period

Source	DF	SS	MS	F	P
Week	2	0.7011	0.35057	3.49	<0.05
Treatment	2	1.5689	0.78445	7.81	<0.005
W*T	4	0.9288	0.23220	2.31	<0.10
Error	39	3.9186	0.10048		
Total	47	7.1175			

Table A12

SUMMARY, FEMALE LIVER WEIGHTS AND MULTIPLE RANGE TEST

Week	Number of Observations	Body Weight (%) for Dose 0.0	0.1	1.0	MRT for Dose: 0	0.1	1.0	Confidence Limits 95%	99%
2	3	5.079	5.153	5.087	a	a	a	±.398	±.530
4	3	4.263	4.537	5.220	a	a	b	∓.398	∓.530
8	3	3.755	4.618	5.234	a	b	c	∓.398	∓.530
16	3	3.467	3.601	4.051	a	ab	b	∓.398	∓.530
28	10	3.362	3.820	3.609	a	b	ab	∓.218	∓.290
32	3	3.058	3.196	3.867	a	a	b	∓ 398	∓.530
40	3	3.502	3.860	3.936	a	a	a	±.398	±.530
Mean	28	3.678	4.039	4.224	a	b	c	±.130	±.173

Table A13

TWO-WAY ANALYSIS OF VARIANCE, MALE SPLEEN AS PERCENT OF BODY WEIGHT

Total Experimental Period

Source	DF	SS	MS	F	P
Week	6	0.53722	0.089537	44.94	<0.0005
Treatment	2	0.00073	0.000365	0.18	n.s.
W*T	12	0.09458	0.007887	3.96	<0.0005
Error	63	0.12553	0.001992		
Total	83	0.75806			

Table A14

SUMMARY, MALE SPLEEN WEIGHTS AND MULTIPLE RANGE TEST

Week	Number of Observations	Spleen Weight (%) for Dose 0.0	0.1	1.0	MRT for Dose: 0	0.1	1.0	Confidence Limits 95%	99%
2	3	0.487	0.380	0.286	c	b	a	±0.052	±0.068
4	3	0.226	0.286	0.345	a	ab	b	±0.052	±0.068
8	3	0.337	0.205	0.203	b	a	a	±0.052	±0.068
16	3	0.151	0.145	0.159	a	a	a	±0.052	±0.068
28	10	0.118	0.134	0.149	a	a	a	±0.028	±0.038
32	3	0.143	0.142	0.147	a	a	a	±0.052	±0.068
40	3	0.132	0.145	0.159	a	a	a	±0.052	±0.068
Mean	28	0.200	0.187	0.192	a	a	a	±0.017	±0.022

Table A15

TWO-WAY ANALYSIS OF VARIANCE, FEMALE SPLEEN AS PERCENT OF BODY WEIGHT

Total Experimental Period

Source	DF	SS	MS	F	P
Week	6	0.25085	0.041809	19.35	<0.0005
Treatment	2	0.01697	0.008485	3.93	<0.025
W*T	12	0.02282	0.001902	0.88	n.s.
Error	63	0.13609	0.002160		
Total	83	0.42673			

Table A16

SUMMARY, FEMALE SPLEEN WEIGHTS AND MULTIPLE RANGE TEST

Week	Number of Observations	Spleen Weight (%) for Dose 0.0	0.1	1.0	MRT for Dose: 0.1	0.1	1.0	Confidence Limits 95%	99%
2	3	0.282	0.318	0.325	a	a	a	±0.054	±0.071
4	3	0.246	0.236	0.331	a	a	b	±0.054	±0.071
8	3	0.205	0.224	0.302	a	a	b	±0.054	±0.071
16	3	0.186	0.179	0.187	a	a	a	±0.054	±0.071
28	10	0.156	0.181	0.176	a	a	a	±0.029	±0.039
32	3	0.146	0.144	0.162	a	a	a	±0.054	±0.071
40	3	0.132	0.140	0.145	a	a	a	±0.054	±0.071
Mean	28	0.184	0.198	0.218	a	ab	b	±0.018	±0.023

Table A17

TWO-WAY ANALYSIS OF VARIANCE, MALE GONADS AS PERCENT OF BODY WEIGHT

Source	DF	SS	MS	F	P
Weeks	6	4.1991	0.69985	39.54	<0.0005
Treatment	2	0.0041	0.00205	0.12	n.s.
W*T	12	0.3445	0.02870	1.62	n.s.
Error	63	1.1150	0.01770		
Total	83	5.6627			

Table A18

SUMMARY, MALE GONAD WEIGHTS AND MULTIPLE RANGE TEST

Week	Number of Observations	Gonad Weight (%) for Dose 0.0	0.1	1.0	MRT for Dose: 0.0	0.1	1.0	Confidence Limits 95%	99%
2	3	1.252	1.092	1.125	a	a	a	±0.154	±0.204
4	3	1.028	0.990	0.903	a	a	a	∓0.154	∓0.204
8	3	0.924	0.712	0.635	b	ab	a	∓0.154	∓0.204
16	3	0.554	0.577	0.501	a	a	a	∓0.154	∓0.204
28	10	0.456	0.529	0.557	a	a	a	∓0.084	∓0.112
32	3	0.358	0.418	0.599	a	ab	b	∓0.154	∓0.204
40	3	0.584	0.459	0.545	a	a	a	∓0.154	∓0.204
Mean	28	0.666	0.644	0.660	a	a	a	±0.050	±0.067

Table A19

TWO-WAY ANALYSIS OF VARIANCE, FEMALE GONADS AS PERCENT OF BODY WEIGHT

	DF	SS	MS	F	P
Weeks	6	0.00451	0.752110	53.44	0.0005
Treatment	2	0.00442	0.002208	1.57	n.s.
W*T	12	0.01294	0.001078	0.77	n.s.
Error	63	0.08867	0.001407		
Total	83	0.15115			

Table A20

SUMMARY, FEMALE GONAD WEIGHTS AND MULTIPLE RANGE TEST

Week	Number of Observations	Gonad Weight (%) for Dose 0.0	0.1	1.0	MRT for Dose: 0.0	0.1	1.0	Cofidence Limits 95%	99%
2	3	0.068	0.124	0.111	a	a	a	±0.043	±0.058
4	3	0.140	0.076	0.114	b	a	ab	±0.043	±0.058
8	3	0.059	0.044	0.073	a	a	a	±0.043	±0.058
16	3	0.044	0.058	0.054	a	a	a	±0.043	±0.058
28	10	0.050	0.062	0.075	a	a	a	±0.024	±0.032
32	3	0.031	0.039	0.045	a	a	a	±0.043	±0.058
40	3	0.027	0.035	0.051	a	a	a	±0.043	±0.058
Mean	28	0.057	0.062	0.075	a	a	a	±0.014	±0.019

Table A21

TWO-WAY ANALYSIS OF VARIANCE, MALE KIDNEYS
AS PERCENT OF BODY WEIGHT

Source	DF	SS	MS	F	P
Weeks	6	2.5337	0.422290	78.41	0.0005
Treatment	2	0.0294	0.014680	2.72	0.10
W*T	12	0.0392	0.003270	0.61	n.s.
Error	63	0.3393	0.005386		
Total	83	2.9415			

Table A22

SUMMARY, MALE KIDNEY WEIGHTS AND MULTIPLE RANGE TEST

Week	Number of Observations	Kidney Weight (%) for Dose			MRT for Dose:			Confidence Limits	
		0.0	0.1	1.0	0.0	0.1	1.0	95%	99%
2	3	1.175	1.184	1.102	a	a	a	±0.085	±0.113
4	3	0.859	0.952	0.867	a	a	a	±0.085	±0.113
8	3	0.795	0.836	0.818	a	a	a	±0.085	±0.113
16	3	0.688	0.650	0.671	a	a	a	±0.085	±0.113
28	10	0.567	0.617	0.610	a	a	a	±0.046	±0.061
32	3	0.600	0.607	0.620	a	a	a	±0.085	±0.113
40	3	0.588	0.633	0.538	a	a	a	±0.085	±0.113
Mean	28	0.707	0.741	0.712	a	a	a	±0.028	±0.037

Table A23

TWO-WAY ANALYSIS OF VARIANCE, FEMALE KIDNEYS AS PERCENT OF BODY WEIGHT

Source	DF	SS	MS	F	P
Week	6	0.90246	0.150410	15.05	<0.0005
Treatment	2	0.11817	0.059080	5.91	<0.005
W*T	12	0.24544	0.020450	2.05	<0.05
Error	63	0.62949	0.009992		
Total	83	1.89556			

Table A24

SUMMARY, FEMALE KIDNEY WEIGHTS AND MULTIPLE RANGE TEST

Week	Number of Observations	Kidney Weight (%) for Dose			MRT for Dose:			Confidence Limits	
		0.0	0.1	1.0	0.0	0.1	1.0	95%	99%
2	3	0.951	1.036	1.022	a	a	a	±0.115	±0.153
4	3	0.868	0.741	0.749	a	a	a	∓0.115	∓0.153
8	3	0.779	0.762	1.040	a	a	b	∓0.115	∓0.153
16	3	0.735	0.685	0.739	a	a	a	∓0.115	∓0.153
28	10	0.622	0.731	0.720	a	b	b	∓0.063	∓0.084
32	3	0.661	0.633	0.736	a	a	a	∓0.115	∓0.153
40	3	0.570	0.669	0.801	a	ab	b	∓0.115	∓0.153
Mean	28	0.711	0.746	0.802	a	a	b	±0.038	±0.050

Table A25

TWO-WAY ANALYSIS OF VARIANCE, MALE HEART AS PERCENT OF BODY WEIGHT

Source	DF	SS	MS	F	P
Week	6	0.34159	0.056932	14.36	<0.0005
Treatment	2	0.00509	0.002545	0.64	n.s.
W*T	12	0.03043	0.002536	0.64	n.s.
Error	63	0.24969	0.003963		
Total	83	0.62630			

Table 26

SUMMARY, MALE HEART WEIGHTS AND MULTIPLE RANGE TEST

Week	Number of Observations	Heart Weight (%) for Dose			MRT for Dose:			Confidence Limits	
		0.0	0.1	1.0	0.0	0.1	1.0	95%	99%
2	3	0.484	0.444	0.458	a	a	a	±0.073	±0.097
4	3	0.373	0.407	0.363	a	a	a	∓0.073	∓0.097
8	3	0.341	0.311	0.315	a	a	a	∓0.073	∓0.097
16	3	0.261	0.251	0.363	a	a	a	∓0.073	∓0.097
28		0.259	0.249	0.278	a	a	a	∓0.040	∓0.053
32	3	0.271	0.283	0.276	a	a	a	∓0.073	∓0.097
40	3	0.276	0.240	0.239	a	a	a	∓0.073	∓0.097
Mean	28	0.307	0.296	0.315	a	a	a	±0.024	±0.032

Table A27

TWO-WAY ANALYSIS OF VARIANCE, FEMALE HEART AS PERCENT OF BODY WEIGHT

Source	DF	SS	MS	F	P
Weeks	6	0.14734	0.024556	8.200	<0.0005
Treatment	2	0.02692	0.013460	4.496	<0.025
W*T	12	0.02179	0.001816	0.61	n.s.
Error	63	0.18859	0.002993		
Total	83	0.38464			

Table A28

SUMMARY, FEMALE HEART WEIGHTS AND MULTIPLE RANGE TEST

Week	Number of Observations	Heart Weight (%) for Dose 0.0	0.1	1.0	MRT for Dose: 0.0	0.1	1.0	Confidence Limits 95%	99%
2	3	0.416	0.440	0.465	a	a	a	±0.063	±0.084
4	3	0.387	0.343	0.389	a	a	a	±0.063	±0.084
8	3	0.354	0.323	0.381	a	a	a	±0.063	±0.084
16	3	0.295	0.328	0.306	a	a	a	±0.063	±0.084
28	10	0.294	0.343	0.351	a	b	b	±0.035	±0.046
32	3	0.278	0.281	0.316	a	a	a	±0.063	±0.084
40	3	0.253	0.312	0.341	a	a	a	±0.063	±0.084
Mean	28	0.317	0.340	0.361	a	ab	b	±0.021	±0.027

Table A29

TWO-WAY ANALYSIS OF VARIANCE, MALE BRAIN
AS PERCENT OF BODY WEIGHT

Source	DF	SS	MS	F	P
Weeks	6	2.44450	0.40741	60.15	0.0005
Treatment	2	0.04344	0.02172	3.21	0.05
W*T	12	0.12405	0.02068		
Error	62	0.41993	0.00677		
Total	82	3.03192			

Table A30

SUMMARY, MALE BRAIN WEIGHTS AND MULTIPLE RANGE TEST

Week	Number of Observations	Brain Weight (g) for Dose 0.0	0.1	1.0	MRT for Dose: 0.0	0.1	1.0	Confidence Limits 95%		99%	
2	3	0.664		0.843	0.911	a	b	b	±0.095		±0.126	
4	3	0.670		0.668	0.646	a	a	a	±0.095		±0.126	
8	3	0.517		0.481	0.631	ab	a	b	±0.095		±0.126	
16	3	0.347		0.287	0.360	a	a	a	±0.095		±0.126	
28	10	0.311	(9)	0.318	0.348	a	a	a	±0.052	(9) ±0.055	±0.069	(9) ±0.073
32	3	0.298		0.352	0.367	a	a	a	±0.095		±0.126	
40	3	0.362		0.292	0.309	a	a	a	±0.095		±0.126	
Mean	28	0.417	(27)	0.493	0.470	a	ab	b	±0.031	(27)±0.036	±0.041	(27)±0.048

Only nine observations were available on male brain dose 0.1 at 28 weeks. This is reflected in wider confidence limits given for nine observations and for the 1.0 dose mean.

Table A31

TWO-WAY ANALYSIS OF VARIANCE, FEMALE BRAIN AS PERCENT OF BODY WEIGHT

Source	DF	SS	MS	F	P
Weeks	6	2.64270	0.44046	68.51	<0.0005
Treatment	2	0.12817	0.06408	9.68	<0.0005
W*T	12	0.18573	0.01548	2.41	<0.025
Error	63	0.04050	0.00643		
Total	83	3.36172			

Table A32

Week	Number of Observations	Brain Weight (%) for Dose 0.0	0.1	1.0	MRT for Dose: 0.0	0.1	1.0	Confidence Limits 95%	99%
2	3	0.970	1.006	1.084	a	a	a	±0.093	±0.123
4	3	1.005	0.810	0.916	b	a	ab	∓0.093	∓0.123
8	3	0.646	0.654	0.897	a	a	b	∓0.093	∓0.123
16	3	0.606	0.602	0.657	a	a	a	∓0.093	∓0.123
28	10	0.496	0.563	0.568	a	a	a	∓0.051	∓0.067
32	3	0.465	0.513	0.536	a	a	a	∓0.093	∓0.123
40	3	0.440	0.443	0.626	a	a	b	∓0.093	∓0.123
Mean	28	0.620	0.633	0.708	a	a	b	±0.030	±0.040

Table A33

TWO-WAY ANALYSIS OF VARIANCE, MALE LUNGS
AS PERCENT OF BODY WEIGHT

Source	DF	MS	SS	F	P
Week	4	0.70711	0.17678	21.72	0.0005
Treatment	2	0.03512	0.01756	2.16	n.s.
W*T	8	0.05929	0.00741	0.91	n.s.
Error	51	0.41500	0.00817		
Total	65	1.21652			

Table A34

SUMMARY, MALE LUNG WEIGHTS AND MULTIPLE RANGE TEST

Week	Number of Observations	Lung Weight (%) for Dose			MRT for Dose:			Confidence Limits	
		0.0	0.1	1.0	0.0	0.1	1.0	95%	99%
8	3	0.765	0.726	0.839	a	a	a	±0.105	±0.139
16	3	0.487	0.593	0.674	a	ab	b	±0.105	±0.139
28	10	0.466	0.504	0.516	a	a	a	±0.057	±0.076
32	3	0.447	0.497	0.477	a	a	a	±0.105	±0.139
40	3	0.472	0.425	0.427	a	a	a	±0.105	±0.139
Mean	22	0.508	0.535	0.564	a	ab	b	±0.039	±0.051

Table A35

TWO-WAY ANALYSIS OF VARIANCE, FEMALE LUNGS
AS PERCENT OF BODY WEIGHT

Source	DF	SS	MS	F	P
Week	4	1.0204	0.25600	8.47	0.0005
Treatment	2	0.2366	0.11830	3.91	0.05
W*T	8	0.3834	0.04792	1.59	n.s.
Error	50	1.5112	0.03022		
Total	64	3.1552			

Table A36

SUMMARY, FEMALE LUNG WEIGHTS AND MULTIPLE RANGE TEST

Week	Number of Observations	Lung Weight (g) for Dose 0.0	0.1	1.0	MRT for Dose: 0.0	0.1	1.0	Confidence Limits 95%		99%	
8	3	0.887	0.847	1.240	a	a	b	±0.202		±0.269	
16	3	0.864	0.720	0.773	a	a	a	±0.202		±0.269	
28	10	0.581	0.778	(9) 0.708	a	b	ab	±0.110	(9) ±0.116	±0.147	(9) ±0.155
32	3	0.510	0.597	0.688	a	a	a	±0.202		±0.269	
40	3	0.470	0.641	0.648	a	a	a	±0.202		±0.269	
Mean	22	0.636	0.736	(21) 0.782	a	ab	b	±0.074	(21) ±0.076	±0.099	(21) ±0.102

Only nine observations were available for female lung dose 1.0 at 28 weeks. This causes wider confidence limits for the mean of nine observations and for the overall mean for 1.0 dose with 21 observations.

APPENDIX B

HEPATIC HISTOLOGIC OBSERVATIONS,

Rats Treated with TCDD for 28 Weeks and Killed Immediately or After 4 or 12 Weeks of Recovery

APPENDIX B

Hepatic Histologic Observations, Rats Treated with TCDD for 28 Weeks and Killed Immediately or After 4 or 12 Weeks of Recovery

28 Weeks

Male Control

Acc. No.

233	Occasional hepatocyte containing large lipid droplets
234	Slight diffuse hepatocellular lipid accumulation
235	More than usual number of random hepatocytes with hyperchromatic nuclei and cytoplasm
236	More than usual number of random hepatocytes with hyperchromatic nuclei and cytoplasm
237	Occasional hepatocyte with large lipid droplets; occasional eosinophilic cytoplasmic inclusion
238	Random hepatocytes with large lipid droplets
239	Normal
240	Normal
241	Mild diffuse hepatocellular lipid accumulation
242	Mild diffuse hepatocellular lipid accumulation

Male 0.1 µg/kg/week

305	Moderate centrilobular hepatocellular fatty change. There has been karyolysis of some centrilobular hepatocytes.
306	Moderate centrilobular fatty change. There also has been some cellular necrosis as there are several small foci of hepatocellular coagulation necrosis with hemorrhage and poly and macrophage response
307	Mild, predominantly centrilobular, fatty change with perhaps loss of a few centrilobular hepatocytes
308	Moderate centrilobular fatty change with a mild Küpffer cell hyperplasia around some central veins
309	Moderate centrilobular fatty change with karyorrhexis seen in an occasional centrilobular hepatocyte
310	Normal
311	Marked centrilobular hepatocellular fatty change. This lesion is extensive in that in a few cases it seems to extend from between the central veins of 2 adjoining lobules. There is occasional round-cell infiltrate, but there is no hemorrhage and most of the nuclei appear to be intact
312	Moderate centrilobular fatty change
313	Slight to mild, predominantly centrilobular, fatty change (would be difficult to differentiate this case from some of the fat accumulation seen in "normals")
314	Normal

Male 1.0 µg/kg/week

163 Random diffuse mild fatty change; otherwise normal
164 Occasional random pigment-filled macrophage; mild, diffuse generally centrilobular, fatty change
165 Moderate centrilobular fatty change; occasional round-cell infiltrate but otherwise essentially normal
166 Moderate centrilobular fatty change
167 Moderate to marked, generally centrilobular, fatty change
168 Moderate centrilobular fatty change
169 Moderate centrilobular fatty change; perhaps slight increase in random cells with hyperchromatic nuclei and cytoplasm
170 Mild to moderate predominantly centrilobular fatty change
171 Marked centrilobular fatty change; there is an occasional centrilobular nucleus undergoing karyorrhexis, but there is no inflammatory reaction
172 Occasional random hepatocyte containing a large lipid droplet; perhaps a small amount of bile duct hyperplasia; otherwise architecture appears normal

28 Weeks

Female Control

268 Normal
269 Normal
270 Normal
271 Normal
272 Normal
273 Slightly above normal incidence of random cells with hyperchromatic cytoplasm and nuclei
274 Normal
275 Normal
276 Normal
277 Normal

Female 0.1 µg/kg/week

338 Occasional hepatocyte with one or two large lipid droplets; slight focal fatty change. Cells at the center of the lobule are slightly smaller than those located more peripherally; considered a very subtle change. This animal could be considered almost normal
339 Perhaps a few more cells present than normal with hyperchromatic nuclei and cytoplasm; otherwise, essentially normal.
340 Other than an occasional centrilobular pigment-laden macrophage, and an occasional cell with slightly more lipid than normal, this liver is essentially normal

341 No section of liver available
342 Centrilobular cells slightly paler staining than those at the periphery; otherwise, essentially normal
343 Normal
344 Mild focal fatty change which occurs in some lobules, while those adjacent are unaffected. The cells at the periphery of the lobule tend to be slightly larger, with larger, hyperchromatic nuclei
345 More cells with hyperchromatic cytoplasm and nuclei are more present than normal. There has been some minor hepatocellular loss from some of the central vein regions; occasional lipid-filled cells located centrilobularly
346 Slight, diffuse fatty change; essentially normal
347 The cytoplasm of the centrilobular hepatocytes is paler than those at the periphery; essentially normal

Female 1.0 µg/kg/week

199 Centrilobular hepatocytes have slightly more lipid in them than usual; occasional pigment-filled macrophage
200 Mild diffuse centrilobular fatty change; slight increase in pigment-containing centrilobular macrophages; perhaps some centrilobular hepatocellular loss
201 Slight to mild multifocal fatty change; slight increase in centrilobular pigment-laden macrophages
202 Mild diffuse centrilobular fatty change; centrilobular region considerably paler staining than peripheral. There appears to have been karyolysis in many centrilobular hepatocytes; slight increase in centrilobular macrophages
204 Mild diffuse fatty change
205 Considerably more hepatocytes with hyperchromatic nuclei and cytoplasm scattered randomly throughout the parenchyma; otherwise essentially normal
206 Many more cells with hyperchromatic nuclei and cytoplasm scattered randomly throughout the parenchyma; occasional multinucleated cell; occasional hepatocyte with more than nomral amount of lipid
207 Occasional random hepatocyte with large lipid droplet; otherwise essentially normal
208 Slight increase in number of hepatocytes with hyperchromatic nuclei and cytoplasm; occasional random hepatocytes with more than normal amounts of lipid

4 Week Recovery

Male Control

243 Occasional hepatocyte containing a large lipid droplet; otherwise normal
244 Normal
245 Other than an occasional random, nonspecific, small lymphoid infiltrate, normal

Male 0.1 µg/kg/week

315 Perhaps slight increase in number of randomly scattered cells with hyperchromatic cytoplasm and nucleus, otherwise normal
316 Slight to mild, generally centrilobular, fatty change
317 Normal

Male 1.0 µg/kg/week

173 Mild diffuse fatty change; almost appears normal
174 Moderate centrilobular fatty change; slight increase in periductule fibrosis; pigment-laden macrophages are seen on rare occasion
175 The centrilobular hepatocytes are very definitely paler staining than those at the periphery of the lobule. There is an occasional hepatocyte in the central part of the lobule which contains a large lipid droplet. It would appear that this liver has been repaired

Female Control

278 Normal
279 Normal
280 Normal

Female 0.1 µg/kg/week

348 Normal
349 A slight increase over control in the number of cells with moderately hyperchromatic nuclei and cytoplasm
350 Occasional minor perivascular lymphoid infiltrate; occasional random cell with slightly hyperchromatic nucleus and cytoplasm in an incidence slightly above that seen in controls; architecture normal; cell size uniform. If this is real and not artifact, it is a very subtle change.

Female 1.0 µg/kg/week

209 There is a marked increase in pigment-containing macrophages particularly in the region of the central vein; there is an occasional hepatocyte containing large lipid droplets; the overall impression is that the centrilobular cells are paler staining and more vaculated than those located in the region of the triad

210 There is a moderate, idffuse, fatty change with some hepatocytes containing rather large lipid droplets. There has been centrilobular hepatocellular loss as there are often empty or blood-filled spaces surrounding the central vein. The cells located in the centrilobular region are generally paler and smaller than those located at the periphery of the lobule. There are many pigment-laden macrophages located mainly in the centrilobular region; occasionaly hepatocytes in the central region are seen to be undergoing karyorrhexis

211 Essentially normal; the centrilobular cells are perhaps slightly paler and the cytoplasm is slightly more vaculated than those cells located at the periphery of the lobule

12 Week Recovery

Male Control

246 Normal
247 Occasional small round-cell infiltrate; there are couple foci of pigment-laden macrophages
248 Normal

Male 0.1 µg/kg/week

318 Moderate centrilobular fatty change. The nuclei in the centrilobular region are much paler than those located at the periphery of the lobule
319 Normal
320 Normal

Male 1.0 µg/kg/week

176 Normal
177 Normal
178 Moderate diffuse fatty change; moderate, predominantly centrilobular, fibrosis. This fobrosis sometimes extends between two central veins or to a major hepatic vein or to a triad area. There are a couple of small foci containing 40 to 50 pleomorphic cells reminescent of early nodular hyperplasia

Female Control

281 Occasional pigment-filled macrophages
282 Normal
283 Normal

Female 0.1 µg/kg/week

351 Occasional pigment-filled macrophages, perhaps more double nucleated cells than usual - not remarkable
352 Occasional round-cell infiltrate, occasional pigment-filled macrophage. Centrilobular region perhaps slightly pale, not remarkable
353 Kupffer cells slightly more prominent than usual, perhaps slight increase in number of double nucleated cells, generally not remarkable

Female 1.0 µg/kg/week

212 Slight increase in the number of hepatocytes containing lipid; occasional multinucleated cell, slight increase in pigment-containing macrophages; variation in cell size slightly greater than normal; all are considered subtle changes; general morphology and architecture is normal
213 More hepatocellular lipid than usual, although not the large lipid droplets seen in the 28 week animals and not necessarily located centrilobularly. Much more variation in nuclear size; these enlarged nuclei tend to be located midzonal and peripheral rather than centrilobular and are distorting the overall architecture slightly. There is a moderate increase in the number of pigment-containing macrophages. Overall impression is that this liver is still undergoing repair
214 Only occasional random cell with increased amount of lipid, perhaps a slight increase in the number pigment-containing macrophages, a slight random increase in nuclear size; architecture is essentially normal
215 Moderate increase in lipid content of the hepatocytes, but these are small droplets. There is moderate bile duct hyperplasia; mild to moderate centrilobular congestion with increase in number of pigment-containing macrophages and some perivascular fibrosis of the central veins. Occasional cells have an increase in nuclear size with a resultant minor alteration in the overall architecture. These changes are considered more than subtle, on the order of mild to moderate